APPENDIX B

SUPPLEMENTARY INFORMATION FOR REFERENCE TOXICITY DATA

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SUPPLEMENTARY INFORMATION FOR REFERENCE TOXICITY DATA

Appendix B contains technical and explanatory notes, and supplementary tables pertaining to the statistical analyses of reference toxicant test results presented in Chapters 3 and 5.

B.1 Acquisition, Selection, and Quality Assurance of Data

Details of data quality assurance and test acceptance are provided in a separate document, available from the EPA Office of Water's Office of Science and Technology ("Whole Effluent Toxicity (WET) Data Test Acceptance and Quality Assurance Protocol"). On request, EPA will also make available a list by laboratory of quality assurance (QA) flags, test dates, toxicant concentration, and summary statistics for the NOEC, EC25, and EC50 estimates and the test endpoints (survival, growth, reproduction, etc.). Laboratories are not named. Data were obtained as data sets from the data base and statistical software packages TOXIS® and TOXCALC® (see Chapter 8 for citations).

TOXIS® software produces an acceptability criterion field code based on the TAC specified by the EPA WET test methods. The tests having "I" (Incomplete) or "F" (Failed) values in this field were eliminated from consideration. TOXCALC® data were examined at the individual test level. The first step, before data entry, consisted of examining the test for TAC from bench sheets. The data were then imported into TOXCALC® for analysis. However, TOXCALC®, unlike TOXIS®, does not generate error codes but issues a warning on the screen. These messages were examined and decisions were made case-by-case following EPA test methods. In the second step, a QA program code was written in SAS® to check the TAC listed in the WET test methods for acute and chronic toxicity tests.

The effect concentration values produced using TOXCALC® or TOXIS®, along with related test information, were exported to spreadsheets and then imported into a SAS® data set. All statistical analyses, other than calculations of effect concentration estimates, were conducted using SAS®. Various data QA tests were conducted. Checks were made to ensure that data were within acceptable concentration-response ranges. Also, the frequency of tests, laboratories, and toxicants were compared for initial and final data sets to ensure that the data were properly imported and exported. Furthermore, TOXIS® effect concentrations having unacceptable error codes such as 905 (i.e., exposure concentrations for LC/EC values unrealistically high due to small slope and estimates well beyond the highest concentration used) and 904 (i.e., non-homogeneity of variance for a Probit estimate) were rejected. The TAC were not verified independently of TOXIS®, although the data used passed the required TAC. Because TOXIS® does not export the qualifier for censored endpoint values (i.e., ">" for greater than and "<" for less than), these qualifiers were later added to cases in which the point estimate equaled the maximum or minimum concentration in the dilution series. The methods having two biological endpoints per test method (e.g., survival and reproduction) had to pass both endpoint TACs to be included in the data analysis.

Non-standard laboratory codes were investigated by follow-up with the data provider; such cases were resolved either by reconfirming the laboratory identity or in a few cases by flagging the data as unusable. Duplicate data sets were identified and eliminated; this involved comparing the test methods, organisms, laboratory codes, test dates, test codes, concentration series, and replicate endpoint means. Concentration units were standardized for each toxicant. Errors in concentration units (e.g., µg versus mg) were identified and resolved. The number of organisms and number of replicates were not used to select or reject tests. For example, the minimum number of replicates was three for Method 1000.0 (which applied to only a few tests, since most tests used four replicates, but some used three) and seven for Method 1002.0 (which was exceptional since most tests used ten replicates).

Only the 20 most recent tests were used if more were submitted. Only laboratories having at least six data points were reported for the toxicants potassium chloride (KCl) and sodium chloride (NaCl) for two common methods: Method 1000.0 (fathead minnow larval survival and growth) and Method 1002.0

(*Ceriodaphnia* survival and reproduction). For other toxicants and methods, the minimum number of data points per laboratory was set at four. The within-laboratory statistics based on only four tests can be imprecise and should be regarded with caution.

In past protocols, the growth and reproduction effect values for the fathead minnow test (Method 1000.0), inland silverside test (Method 1006.0), and mysid test (Method 1007.0) were determined by dividing the weight or reproduction by the number of survivors. In contrast, the currently promulgated methods require that the weight or reproduction values be divided by the original (starting) number of organisms. All such results herein were calculated as currently required, using the weight or reproduction divided by the original number of organisms.

Note that data for Method 1016.0 (purple urchin fertilization test) and Method 1017.0 (sand dollar fertilization) included three different test methods with primary method differences including different sperm-egg ratios, sperm collection procedures, and sperm exposure time. This method has since been standardized and included in the West Coast chronic marine test methods manual (USEPA 1995).

A large percentage of data from a few laboratories was censored (i.e., recorded as “<” or “>”) because the effect concentration was outside the range of the concentration series. In some cases, the data were censored because of the number or range of toxicant concentrations tested. When many data are censored, a reversal in the most sensitive endpoint can occur. For example, in the data for Method 1006.0 (*Menidia beryllina* larval survival and growth test), the NOEC for the survival endpoint indicated a more sensitive response than the sublethal endpoint for some tests.

B.2 Summary Statistics for IC25, LC50, and NOEC

B.2.1 Within-Laboratory Variability of EC25, EC50, and NOEC

Test data were not screened for outliers as provided for in ASTM Practices D2777 and E691 (ASTM 1992, 1998). Thus, maximum and minimum values for the laboratory statistics summarized in Tables B-1 through B-6 may be distorted by outliers. Therefore, EPA concluded that the maximum and minimum values are not necessarily reliable and has not reported them in these tables. EPA recommends that the 10^{th} and 90^{th} percentiles reported in Tables B-1 through B-6 be used to characterize the range of test variability.

Tables B-1 through B-3 show percentiles of the within-laboratory coefficients of variation (CVs) for EC25, EC50, and NOEC for all methods in the variability data set. However, when a method is represented by few laboratories, this summary cannot be considered typical or representative. When there were fewer than ten laboratories for a method, the 10^{th} and 90^{th} percentiles could not be estimated in an unbiased manner. Columns P10 and P90 show the minimum and maximum in such cases. Similarly, when there were fewer than four laboratories, columns P10 and P25 show the minimum and columns P75 and P90 show the maximum. An unbiased estimate of the median is always shown.

These percentiles are found by interpolation between two sample order statistics. The k^{th} sample order statistic has an expected probability estimated by $P_k = (k - 0.375)/(N + 0.25)$. Linear interpolation between two order statistics (X_k and X_{k+1}) having expected probabilities $P_k < P < P_{k+1}$ provides the estimate of the P^{th} quantile.

Tables B-4 through B-6 summarize variation across laboratories for the within-laboratory normal ratio of extremes for the EC25, EC50, and NOEC estimates. Instead of using the ratio of largest-to-smallest observations, which is vulnerable to outliers, the ratio of the 90^{th} to the 10^{th} percentiles (symbolized P90:P10) was used to provide some robustness to outliers. This ratio is a measure of variability in terms of concentration ratio. About 80 percent of observations are expected to fall between these percentiles. Thus, if P90:P10 equals 4, about 80 percent of observations are expected to fall within a dilution ratio of 4 (e.g., 0.25 mg/L to 1.00 mg/L).

The ratio is dimensionless and a more useful measure of the "range" of test results than the concentration range. For example, NOECs may vary at one laboratory between 0.5 mg/L and 2.0 mg/L (giving a range of 1.5 mg/L) and at another laboratory between 0.25 mg/L and 1.0 mg/L (giving a range of 0.75 mg/L), yet both NOECs span two standard concentrations having a ratio of 1:4. Also, using a ratio allows direct comparison among different toxicants having different concentration units. Further, toxicity tests often require a log scale (that is, a ratio scale) of concentration to provide an approximately linear curve of endpoint response (Collett 1991). Environment Canada (2000) expects that plotting and statistical estimation for WET tests will employ a logarithmic scale. In EPA publications, logarithmic (constant-ratio) graphical scales are used for concentrations (USEPA 1994a,1994b).

Tables B-4 through B-6 provide an easy way to quantify the ratio among effect concentrations expected for 80 percent of tests. For example, in Table B-6 under the NOEC for the growth endpoint of Method 1000.0, the median laboratory has a ratio of 2.0. This means that for half of the laboratories, repeated reference toxicant tests gave NOECs, 80 percent of which differed by no more than one standard dilution. That is, most NOECs occurred at only one concentration or at two adjacent concentrations at half of the laboratories. Note that most tests used 1:2 dilutions, so for the NOEC, the only exact ratios possible for each test are 1:1, 1:2, 1:4, 1:8, and 1:16. Thus, for NOECs, the results presented in the tables may be interpreted by rounding to these ratios.

The ratios P90:P10 in Tables B-4 through B-6 can be summarized as follows. For the NOEC in most of the promulgated WET methods, 75 percent of laboratories achieve a ratio of no more than 1:4, and half of the laboratories routinely achieve ratios of 1:1 or 1:2. For the LC50 (survival endpoint) for most methods, 75 percent of laboratories have ratios no more than 1:3, and half the laboratories have ratios no more than 1:2. For the IC25 (growth and reproduction endpoints), 75 percent of laboratories have ratios no more than 1:4, and half of laboratories have ratios no more than 1:2.5. The ratio for acute methods is usually somewhat less than that for chronic methods.

Note that two laboratories having the same ratio P90:P10 do not necessarily have similar NOECs; between-laboratory variation also occurs. For example, consider three laboratories that reported data for the growth endpoint of Method 1000.0 tested with NaCl. Each has a ratio P90:P10 of 2.0. One laboratory reported 11 tests, with the NOEC ranging from 0.4 mg/L to 3.2 mg/L. The 10^{th} and 90^{th} percentile estimates were 1.6 and 3.2. A second laboratory reported 8 tests, with the NOEC ranging from 1.0 mg/L to 2.0 mg/L. The 10^{th} and 90^{th} percentile estimates were 1.0 and 2.0. A third laboratory reported 12 tests, with the NOEC ranging from 1.0 mg/L to 4.0 mg/L. The 10^{th} and 90^{th} percentile estimates were 1.0 and 2.0.

B.2.2 Between-Laboratory Variability of EC25, EC50, and NOEC

The estimates of within- and between-laboratory variability for WET tests in Table 3-5 (Chapter 3) are based on Type-I analysis of variance and expected mean squares for random effects. Within-laboratory variability is estimated as the square root of the error mean square (column "Within-lab σ_w"), that is, the pooled standard deviation for all tests and all laboratories available for a given method, toxicant, and endpoint. Column "Between-lab σ_b" is the square root of the between-laboratory variance term, calculated as shown below. The column headed "Mean" shows the mean of the (unweighted) laboratory means. Sample sizes (numbers of laboratories) are insufficient for credible estimates of between-laboratory variability for most methods. The expected mean squares assume that the population of laboratories is large. Finite population estimates would be more accurate for some combinations of method and toxicant.

Table B-1. Percentiles of the Within-Laboratory Values of CV for EC25

Test Method[a]	Test Method No.[b]	End-point[c]	No. of Labs	CV				
				P10	P25	P50	P75	P90
Chronic, Promulgated								
Fathead Minnow Larval Survival & Growth	1000.0	G	19	0.12	0.21	0.26	0.38	0.45
Fathead Minnow Larval Survival & Growth	1000.0	S	16	0.03	0.11	0.22	0.32	0.52
Ceriodaphnia (Cd) Survival & Reproduction	1002.0	R	33	0.08	0.17	0.27	0.45	0.62
Ceriodaphnia (Cd) Survival & Reproduction	1002.0	S	25	0.07	0.11	0.23	0.41	0.81
Green Alga (*Selenastrum*)[d] Growth	1003.0	G	6	0.02	0.25	0.26	0.39	0.51
Sheepshead Minnow Larval Survival & Growth	1004.0	G	5	0.03	0.09	0.13	0.14	0.18
Sheepshead Minnow Larval Survival & Growth	1004.0	S	2	0.15	0.15	0.16	0.17	0.17
Inland Silverside Larval Survival & Growth	1006.0	G	16	0.05	0.18	0.27	0.43	0.55
Inland Silverside Larval Survival & Growth	1006.0	S	13	0.15	0.22	0.35	0.42	0.62
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	R	4	0.22	0.03	0.38	0.41	0.42
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	G	10	0.21	0.24	0.28	0.32	0.04
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	S	7	0.17	0.17	0.21	0.28	0.32
Red Macroalga *(Champia parvula)* Reprod	1009.0	R	2	0.58	0.58	0.58	0.59	0.59
West Coast								
Topsmelt Larval Survival & Growth	1010.0	G	1	0.25	0.25	0.25	0.25	0.25
Topsmelt Larval Survival & Growth	1010.0	S	1	0.20	0.20	0.20	0.20	0.20
Pacific Oyster Embryo-Larval Survival & Dev.	1012.0	D	1	0.25	0.25	0.25	0.25	0.25
Mussel Embryo-Larval Survival & Dev.	1013.0	D	3	0.14	0.14	0.27	0.42	0.42
Red Abalone Larval Development	1014.0	D	10	0.13	0.15	0.25	0.35	0.36
Sea Urchin Fertilization	1016.0	F	12	0.18	0.26	0.41	0.58	0.68
Sand Dollar Fertilization	1017.0	F	7	0.25	0.35	0.43	0.51	0.60
Giant Kelp Germination & Germ-Tube Length	1018.0	G_e	11	0.33	0.34	0.40	0.43	0.60
Giant Kelp Germination & Germ-Tube Length	1018.0	L	11	0.22	0.25	0.31	0.36	0.36
Acute								
Fathead Minnow Larval Survival	2000.0	S	7	0.05	0.09	0.15	0.21	0.44
Ceriodaphnia (Cd) Survival	2002.0	S	8	0.04	0.09	0.10	0.19	0.33
Sheepshead Minnow Survival	2004.0	S	3	0.08	0.08	0.13	0.46	0.46
Inland Silverside Larval Survival	2006.0	S	4	0.03	0.09	0.20	0.40	0.55
Mysid (Ab) Survival	2007.0	S	1	0.26	0.26	0.26	0.26	0.26
Mysid (Hc) Survival	2011.0	S	1	0.20	0.20	0.20	0.20	0.20
Rainbow Trout Survival	2019.0	S	1	0.11	0.11	0.11	0.11	0.11
Daphnia (Dm) Survival	2021.0	S	1	0.19	0.19	0.19	0.19	0.19
Daphnia (Dp) Survival	2022.0	S	3	0.06	0.06	0.41	0.48	0.48

[a] Cd = *Ceriodaphnia dubia*, Ab = *Americamysis (Mysidopsis) bahia*, Hc = *Holmesimysis costata*, Dm = *Daphnia magna*, Dp = *Daphnia pulex*

[b] EPA did not assign method numbers for acute methods in EPA/600/4-90/027F. The numbers assigned here were created for use in this document and in related materials and data bases.

[c] D = development, F = fertilization, G = growth, G_e = Germination, L = length, R = reproduction or fecundity, S = survival

[d] Genus and species recently changed to *Raphidocelis subcapitata*.

Table B-2. Percentiles of the Within-Laboratory Values of CV for EC50[a]

Test Method[b]	Test Method No.[c]	End-point[d]	No. of Labs	CV				
				P10	P25	P50	P75	P90
Chronic, Promulgated								
Fathead Minnow Larval Survival & Growth	1000.0	G	19	0.10	0.15	0.24	0.26	0.46
Fathead Minnow Larval Survival & Growth	1000.0	S	19	0.12	0.15	0.23	0.31	0.44
Ceriodaphnia (Cd) Survival & Reproduction	1002.0	R	33	0.06	0.12	0.23	0.29	0.46
Ceriodaphnia (Cd) Survival & Reproduction	1002.0	S	33	0.04	0.10	0.16	0.29	0.46
Green Alga (*Selenastrum*)[e] Growth	1003.0	G	9	0.16	0.19	0.27	0.30	0.63
Sheepshead Minnow Larval Survival & Growth	1004.0	G	5	0.02	0.04	0.06	0.11	0.13
Sheepshead Minnow Larval Survival & Growth	1004.0	S	5	0.02	0.07	0.08	0.12	0.13
Inland Silverside Larval Survival & Growth	1006.0	G	16	0.03	0.16	0.26	0.37	0.50
Inland Silverside Larval Survival & Growth	1006.0	S	16	0.05	0.16	0.28	0.35	0.49
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	R	4	0.06	0.17	0.30	0.37	0.43
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	G	10	0.15	0.19	0.22	0.27	0.31
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	S	10	0.12	0.16	0.26	0.27	0.28
Red Macroalga *(Champia parvula)* Reprod	1009.0	R	2	0.35	0.35	0.36	0.38	0.38
West Coast Methods								
Topsmelt Larval Survival & Growth	1010.0	G	1	0.25	0.25	0.25	0.25	0.25
Topsmelt Larval Survival & Growth	1010.0	S	1	0.17	0.17	0.17	0.17	0.17
Pacific Oyster Embryo-Larval Survival & Dev.	1012.0	D	1	0.21	0.21	0.21	0.21	0.21
Mussel Embryo-Larval Survival & Dev.	1013.0	D	3	0.25	0.25	0.35	0.35	0.35
Red Abalone Larval Development	1014.0	D	10	0.13	0.16	0.21	0.28	0.33
Sea Urchin Fertilization	1016.0	F	12	0.24	0.30	0.35	0.52	0.61
Sand Dollar Fertilization	1017.0	F	7	0.28	0.33	0.34	0.50	0.79
Giant Kelp Germination & Germ-Tube Length	1018.0	G_e	11	0.18	0.20	0.30	0.37	0.40
Giant Kelp Germination & Germ-Tube Length	1018.0	L	11	0.17	0.18	0.25	0.32	0.32
Acute								
Fathead Minnow Larval Survival	2000.0	S	21	0.08	0.10	0.16	0.19	0.33
Ceriodaphnia (Cd) Survival	2002.0	S	23	0.06	0.11	0.19	0.29	0.34
Sheepshead Minnow Survival	2004.0	S	5	0.11	0.12	0.14	0.21	0.37
Inland Silverside Larval Survival	2006.0	S	5	0.07	0.15	0.16	0.21	0.44
Mysid (Ab) Survival	2007.0	S	3	0.17	0.17	0.25	0.26	0.26
Mysid (Hc) Survival	2011.0	S	2	0.27	0.27	0.30	0.34	0.34
Rainbow Trout Survival	2019.0	S	1	0.23	0.23	0.23	0.23	0.23
Daphnia (Dm) Survival	2021.0	S	5	0.05	0.07	0.22	0.24	0.46
Daphnia (Dp) Survival	2022.0	S	6	0.15	0.19	0.21	0.27	0.48

[a] EC50 is a more general term than LC50 and may be used to represent an LC50 endpoint (such as survival).
[b] Cd = *Ceriodaphnia dubia*, Ab = *Americamysis (Mysidopsis) bahia*, Hc = *Holmesimysis costata*, Dm = *Daphnia magna*, Dp = *Daphnia pulex*
[c] See footnote b on Table B-1.
[d] D = development, F = fertilization, G = growth, G_e = Germination, L = length, R = reproduction or fecundity, S = survival
[e] Genus and species recently changed to *Raphidocelis subcapitata*.

Table B-3. Percentiles of the Within-Laboratory Values of CV for NOEC

Test Method[a]	Test Method No.[b]	End-point[c]	No. of Labs	CV				
				P10	P25	P50	P75	P90
Chronic, Promulgated								
Fathead Minnow Larval Survival & Growth	1000.0	G	19	0	0.22	0.37	0.53	0.65
Fathead Minnow Larval Survival & Growth	1000.0	S	19	0.13	0.26	0.39	0.48	0.59
Ceriodaphnia (Cd) Survival & Reproduction	1002.0	R	33	0.20	0.25	0.33	0.49	0.60
Ceriodaphnia (Cd) Survival & Reproduction	1002.0	S	33	0.09	0.21	0.30	0.43	0.55
Green Alga (*Selenastrum*)[d] Growth	1003.0	G	9	0.30	0.40	0.46	0.56	0.82
Sheepshead Minnow Larval Survival & Growth	1004.0	G	5	0.20	0.34	0.40	0.44	0.52
Sheepshead Minnow Larval Survival & Growth	1004.0	S	5	0	0.14	0.18	0.24	0.38
Inland Silverside Larval Survival & Growth	1006.0	G	16	0.14	0.31	0.46	0.57	0.63
Inland Silverside Larval Survival & Growth	1006.0	S	16	0.19	0.30	0.42	0.55	0.66
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	R	4	0	0.17	0.36	0.40	0.41
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	G	10	0.22	0.35	0.39	0.43	0.67
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	S	10	0.13	0.28	0.33	0.38	0.41
Red Macroalga *(Champia parvula)* Reprod	1009.0	R	2	0.85	0.85	1.00	1.16	1.16
West Coast Methods								
Topsmelt Larval Survival & Growth	1010.0	G	1	0.31	0.31	0.31	0.31	0.31
Topsmelt Larval Survival & Growth	1010.0	S	1	0.42	0.42	0.42	0.42	0.42
Pacific Oyster Embryo-Larval Survival & Dev.	1012.0	D	1	0.45	0.45	0.45	0.45	0.45
Mussel Embryo-Larval Survival & Dev.	1013.0	D	3	0	0	0.39	0.43	0.43
Red Abalone Larval Development	1014.0	D	10	0.24	0.25	0.29	0.31	0.38
Sea Urchin Fertilization	1016.0	F	12	0.31	0.40	0.50	0.69	0.76
Sand Dollar Fertilization	1017.0	F	7	0.40	0.41	0.53	0.75	0.81
Giant Kelp Germination & Germ-Tube Length	1018.0	G_e	11	0.36	0.40	0.54	0.65	0.81
Giant Kelp Germination & Germ-Tube Length	1018.0	L	11	0.39	0.48	0.59	0.68	0.76
Acute								
Fathead Minnow Larval Survival	2000.0	S	21	0.15	0.18	0.22	0.34	0.61
Ceriodaphnia (Cd) Survival	2002.0	S	23	0.07	0.18	0.35	0.41	0.57
Sheepshead Minnow Survival	2004.0	S	3	0.0	0	0.31	0.33	0.33
Inland Silverside Larval Survival	2006.0	S	5	0.0	0	0.33	0.35	0.72
Mysid (Ab) Survival	2007.0	S	3	0.29	0.29	0.38	0.43	0.43
Mysid (Hc) Survival	2011.0	S	2	0.21	0.21	0.26	0.31	0.31
Rainbow Trout Survival	2019.0	S	1	0.35	0.35	0.35	0.35	0.35
Daphnia (Dm) Survival	2021.0	S	5	0	0.09	0.36	0.47	0.83
Daphnia (Dp) Survival	2022.0	S	6	0.20	0.21	0.38	0.61	0.67

[a] Cd = *Ceriodaphnia dubia*, Ab = *Americamysis (Mysidopsis) bahia*, Hc = *Holmesimysis costata*, Dm = *Daphnia magna*, Dp = *Daphnia pulex*

[b] See footnote b on Table B-1.

[c] D = development, F = fertilization, G = growth, G_e = germination, L = length, R = reproduction or fecundity, S = survival

[d] Genus and species recently changed to *Raphidocelis subcapitata*.

Table B-4. Variation Across Laboratories in the Within-Laboratory Value of P90:P10 for EC25

Test Method[a]	Test Method No.[b]	End-point[c]	No. of Labs	CV				
				P10	P25	P50	P75	P90
Chronic, Promulgated								
Fathead Minnow Larval Survival & Growth	1000.0	G	19	1.3	1.7	2.1	3.6	4.1
Fathead Minnow Larval Survival & Growth	1000.0	S	16	1.0	1.3	1.7	2.3	3.5
Ceriodaphnia (Cd) Survival & Reproduction	1002.0	R	33	1.2	1.4	2.2	3.6	6.3
Ceriodaphnia (Cd) Survival & Reproduction	1002.0	S	25	1.1	1.3	1.6	2.6	4.8
Green Alga (*Selenastrum*)[d] Growth	1003.0	G	6	1.7	1.8	2.0	2.5	3.8
Sheepshead Minnow Larval Survival & Growth	1004.0	G	5	1.1	1.1	1.4	1.4	1.4
Sheepshead Minnow Larval Survival & Growth	1004.0	S	2	1.3	1.3	1.3	1.3	1.3
Inland Silverside Larval Survival & Growth	1006.0	G	16	1.1	1.5	2.0	2.5	4.2
Inland Silverside Larval Survival & Growth	1006.0	S	13	1.3	1.7	2.2	3.2	4.3
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	R	4	1.7	2.1	2.4	2.7	2.9
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	G	10	1.4	1.8	2.2	2.6	3.0
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	S	7	1.5	1.5	1.8	2.4	2.5
Red Macroalga *(Champia parvula)* Reprod	1009.0	R	2	6.7	6.7	10.2	13.7	13.7
West Coast								
Topsmelt Larval Survival & Growth	1010.0	G	1	1.7	1.7	1.7	1.7	1.7
Topsmelt Larval Survival & Growth	1010.0	S	1	1.8	1.8	1.8	1.8	1.8
Pacific Oyster Embryo-Larval Survival & Dev.	1012.0	D	1	2.0	2.0	2.0	2.0	2.0
Mussel Embryo-Larval Survival & Dev.	1013.0	D	3	1.4	1.4	2.2	4.0	4.0
Red Abalone Larval Development	1014.0	D	10	1.3	1.5	2.0	2.9	3.1
Sea Urchin Fertilization	1016.0	F	12	1.6	1.8	3.0	6.7	14.9
Sand Dollar Fertilization	1017.0	F	7	2.4	3.1	3.8	3.9	6.1
Giant Kelp Germination & Germ-Tube Length	1018.0	G_e	11	2.1	2.1	3.3	4.1	5.9
Giant Kelp Germination & Germ-Tube Length	1018.0	L	11	1.7	1.8	2.3	2.5	3.1
Acute								
Fathead Minnow Larval Survival	2000.0	S	7	1.1	1.2	1.4	1.5	3.7
Ceriodaphnia (Cd) Survival	2002.0	S	8	1.1	1.1	1.3	1.4	1.6
Sheepshead Minnow Survival	2004.0	S	3	1.2	1.2	1.3	5.2	5.2
Inland Silverside Larval Survival	2006.0	S	4	1.0	1.3	1.7	2.6	3.4
Mysid (Ab) Survival	2007.0	S	1	1.7	1.7	1.7	1.7	1.7
Mysid (Hc) Survival	2011.0	S	1	1.5	1.5	1.5	1.5	1.5
Rainbow Trout Survival	2019.0	S	1	1.2	1.2	1.2	1.2	1.2
Daphnia (Dm) Survival	2021.0	S	1	1.9	1.9	1.9	1.9	1.9
Daphnia (Dp) Survival	2022.0	S	3	1.1	1.1	2.5	2.8	2.8

[a] Cd = *Ceriodaphnia dubia*, Ab = *Americamysis (Mysidopsis) bahia*, Hc = *Holmesimysis costata*, Dm = *Daphnia magna*, Dp = *Daphnia pulex*

[b] See footnote b on Table B-1.

[c] D = development, F = fertilization, G = growth, G_e = germination, L = length, R = reproduction or fecundity, S = survival

[d] Genus and species recently changed to *Raphidocelis subcapitata*.

Table B-5. Variation Across Laboratories in the Within-Laboratory Value of P90:P10 for EC50[a]

Test Method[b]	Test Method No.[c]	End-point[d]	No. of Labs	CV P10	CV P25	CV P50	CV P75	CV P90
Chronic, Promulgated								
Fathead Minnow Larval Survival & Growth	1000.0	G	19	1.3	1.5	1.8	2.4	3.3
Fathead Minnow Larval Survival & Growth	1000.0	S	19	1.4	1.5	1.8	2.3	3.0
Ceriodaphnia (Cd) Survival & Reproduction	1002.0	R	33	1.2	1.3	1.7	2.3	3.7
Ceriodaphnia (Cd) Survival & Reproduction	1002.0	S	33	1.1	1.3	1.5	2.2	3.5
Green Alga (*Selenastrum*)[e] Growth	1003.0	G	9	1.2	1.5	1.7	2.4	9.4
Sheepshead Minnow Larval Survival & Growth	1004.0	G	5	1.0	1.1	1.1	1.2	1.3
Sheepshead Minnow Larval Survival & Growth	1004.0	S	5	1.0	1.1	1.1	1.2	1.3
Inland Silverside Larval Survival & Growth	1006.0	G	16	1.1	1.5	1.8	2.7	3.5
Inland Silverside Larval Survival & Growth	1006.0	S	16	1.2	1.5	1.9	2.8	2.9
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	R	4	1.2	1.5	1.9	2.4	2.9
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	G	10	1.4	1.5	1.8	2.2	2.4
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	S	10	1.4	1.6	1.9	2.0	2.3
Red Macroalga *(Champia parvula)* Reprod	1009.0	R	2	2.3	2.3	4.9	7.6	7.6
West Coast								
Topsmelt Larval Survival & Growth	1010.0	G	1	1.7	1.7	1.7	1.7	1.7
Topsmelt Larval Survival & Growth	1010.0	S	1	1.5	1.5	1.5	1.5	1.5
Pacific Oyster Embryo-Larval Survival & Dev.	1012.0	D	1	2.0	2.0	2.0	2.0	2.0
Mussel Embryo-Larval Survival & Dev.	1013.0	D	3	2.0	2.0	2.0	2.8	2.8
Red Abalone Larval Development	1014.0	D	10	1.4	1.4	1.8	2.4	2.6
Sea Urchin Fertilization	1016.0	F	12	1.8	2.0	2.9	4.2	6.5
Sand Dollar Fertilization	1017.0	F	7	2.4	2.6	2.8	4.4	6.0
Giant Kelp Germination & Germ-Tube Length	1018.0	G_e	11	1.7	1.8	2.1	3.3	3.6
Giant Kelp Germination & Germ-Tube Length	1018.0	L	11	1.6	1.6	1.8	2.5	2.7
Acute								
Fathead Minnow Larval Survival	2000.0	S	21	1.2	1.3	1.5	1.7	2.6
Ceriodaphnia (Cd) Survival	2002.0	S	23	1.1	1.2	1.7	2.0	2.4
Sheepshead Minnow Survival	2004.0	S	5	1.1	1.2	1.4	1.7	2.8
Inland Silverside Larval Survival	2006.0	S	5	1.2	1.4	1.6	1.7	2.7
Mysid (Ab) Survival	2007.0	S	3	1.7	1.7	2.1	2.1	2.1
Mysid (Hc) Survival	2011.0	S	2	1.8	1.8	2.5	3.1	3.1
Rainbow Trout Survival	2019.0	S	1	1.8	1.8	1.8	1.8	1.8
Daphnia (Dm) Survival	2021.0	S	5	1.2	1.2	1.8	2.2	4.1
Daphnia (Dp) Survival	2022.0	S	6	1.4	1.5	1.9	2.1	2.2

[a] EC50 is a more general term than LC50 and may be used to represent an LC50 endpoint (such as survival).
[b] Cd = *Ceriodaphnia dubia*, Ab = *Americamysis (Mysidopsis) bahia*, Hc = *Holmesimysis costata*, Dm = *Daphnia magna*, Dp = *Daphnia pulex*
[c] See footnote b on Table B-1.
[d] D = development, F = fertilization, G = growth, G_e = germination, L = length, R = reproduction or fecundity, S = survival
[e] Genus and species recently changed to *Raphidocelis subcapitata*.

Table B-6. Variation Across Laboratories in the Within-Laboratory Value of P90:P10 for NOEC

Test Method[a]	Test Method No.[b]	End-point[c]	No. of Labs	CV				
				P10	P25	P50	P75	P90
Chronic, Promulgated								
Fathead Minnow Larval Survival & Growth	1000.0	G	19	1.0	1.5	2.0	4.2	8.0
Fathead Minnow Larval Survival & Growth	1000.0	S	19	1.0	1.7	2.0	4.0	5.0
Ceriodaphnia (Cd) Survival & Reproduction	1002.0	R	33	1.3	1.9	2.2	4.0	4.0
Ceriodaphnia (Cd) Survival & Reproduction	1002.0	S	33	1.0	1.5	2.0	3.0	5.3
Green Alga (*Selenastrum*)[d] Growth	1003.0	G	9	1.8	2.0	2.7	4.0	10.0
Sheepshead Minnow Larval Survival & Growth	1004.0	G	5	1.3	2.0	2.0	4.0	4.0
Sheepshead Minnow Larval Survival & Growth	1004.0	S	5	1.0	1.0	1.3	2.0	2.0
Inland Silverside Larval Survival & Growth	1006.0	G	16	1.3	2.0	4.0	4.2	7.8
Inland Silverside Larval Survival & Growth	1006.0	S	16	1.8	2.0	2.9	4.0	4.1
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	R	4	1.0	1.5	2.0	2.0	2.0
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	G	10	1.9	2.0	2.0	4.0	7.6
Mysid (Ab) Survival, Growth, & Fecundity	1007.0	S	10	1.4	2.0	2.0	2.0	3.4
Red Macroalga *(Champia parvula)* Reprod	1009.0	R	2	5.6	5.6	12.8	20.0	20.0
West Coast								
Topsmelt Larval Survival & Growth	1010.0	G	1	1.8	1.8	1.8	1.8	1.8
Topsmelt Larval Survival & Growth	1010.0	S	1	3.2	3.2	3.2	3.2	3.2
Pacific Oyster Embryo-Larval Survival & Dev.	1012.0	D	1	4.0	4.0	4.0	4.0	4.0
Mussel Embryo-Larval Survival & Dev.	1013.0	D	3	1.0	1.0	3.2	4.0	4.0
Red Abalone Larval Development	1014.0	D	10	1.2	1.8	1.8	1.8	3.2
Sea Urchin Fertilization	1016.0	F	12	1.8	2.0	4.0	6.9	9.4
Sand Dollar Fertilization	1017.0	F	7	2.1	3.1	4.0	6.0	17.8
Giant Kelp Germination & Germ-Tube Length	1018.0	G_e	11	1.8	2.3	3.2	5.7	5.7
Giant Kelp Germination & Germ-Tube Length	1018.0	L	11	3.1	3.1	5.6	5.7	10.0
Acute								
Fathead Minnow Larval Survival	2000.0	S	21	1.3	1.5	1.6	2.0	4.0
Ceriodaphnia (Cd) Survival	2002.0	S	23	1.0	1.3	2.0	3.3	5.0
Sheepshead Minnow Survival	2004.0	S	3	1.0	1.0	2.0	2.0	2.0
Inland Silverside Larval Survival	2006.0	S	5	1.0	1.0	1.8	2.0	4.0
Mysid (Ab) Survival	2007.0	S	3	2.7	2.7	3.2	5.0	5.0
Mysid (Hc) Survival	2011.0	S	2	1.8	1.8	1.9	2.1	2.1
Rainbow Trout Survival	2019.0	S	1	2.0	2.0	2.0	2.0	2.0
Daphnia (Dm) Survival	2021.0	S	5	1.0	1.3	2.0	4.0	6.1
Daphnia (Dp) Survival	2022.0	S	6	1.3	1.7	2.0	2.0	10.0

[a] Cd = *Ceriodaphnia dubia*, Ab = *Americamysis (Mysidopsis) bahia*, Hc = *Holmesimysis costata*, Dm = *Daphnia magna*, Dp = *Daphnia pulex*

[b] See footnote b on Table B-1.

[c] D = development, F = fertilization, G = growth, G_e = germination, L = length, R = reproduction or fecundity, S = survival

[d] Genus and species recently changed to *Raphidocelis subcapitata*.

Estimation formulas were:

Expected mean square for error (within-laboratory): σ_w^2

Expected mean square between-laboratories: $\sigma_w^2 + U\,\sigma_b^2$

$U = [\sum n_i - (\sum n_i^2 / \sum n_i)] / (L-1)$

L is the number of laboratories and n_i the number of tests within the i^{th} laboratory (i = 1, ... L).

B.3 Variability of Endpoint Measurements

Dunnett's critical value, needed for the minimum significant difference (MSD), was computed using the SAS function "PROBMC," for a one-sided test at the 0.95 level ($\alpha = 0.05$). Note that Dunnett's test can be applied when the number of replicates differs among treatments (Dunnett 1964), and that the SAS function "PROBMC" can calculate an appropriate critical value for the case of unequal replication.

The MSD was calculated for sublethal endpoints using untransformed values of "growth" (larval biomass) and "reproduction" (number of offspring in the *Ceriodaphnia* test, or cells per mL in the *Selenastrum* test), and for lethal endpoints using the arc sine transform (arc sine ($\sqrt{p}$)) of the proportion surviving. The CV was calculated for all endpoints using the untransformed mean control response.

Tables B-7 and B-8 show percentiles of CV and of the percent minimum significant difference (PMSD), which is [100×MSD/(control mean)]. These are the sample percentiles for all tests in the data set (see row "No. of tests"). Data for all laboratories and toxicants for a given method and endpoint were combined.

Methods in Tables B-1 through B-3 that are represented by fewer than three laboratories or fewer than 20 tests are not shown in Tables B-7 and B-8, because characterizing method variability using so few tests and laboratories would be inadvisable.[1]

B.4 Test Power to Detect Toxic Effects

Power can be characterized only by repeated testing. It is an attribute, not of a single test, but of a sequence of many tests conducted under similar conditions and the same test design. Therefore, the sample averages for each laboratory's data set are used in this analysis to characterize each laboratory. The key parameters required were the (a) mean endpoint response in the control (growth, reproduction, survival) and (b) the mean value of the error mean square (EMS) for tests.

Power is reported in this section for single two-sample, one-sided t-tests at $1-\alpha = 0.95$, and for a set of k such tests (comparing k treatments to a control) at level $1 - \alpha/k = 1 - 0.05/k$. Some permitting authorities may require a comparison between control and the receiving water concentration, which requires a two-sample, one-sided test. Others may require the multiple comparisons procedure described in the EPA WET methods (Dunnett's or Steel's tests, one-sided, with $\alpha = 0.05$). The power of Dunnett's procedure (using $\alpha = 0.05$ as recommended in EPA effluent test methods) will fall between the power of the one-sided, two-sample t-test with $\alpha = 0.05$ and that with $\alpha = 0.05/k$, when k toxicant concentrations are compared to a control. The power of Steel's procedure will be related to and should usually increase with the power of Dunnett's procedure and the t-tests, so the following tables will also provide an inexact guide to power achieved by the nonparametric test.

Tables B-9 through B-13 illustrate the ability of the sublethal endpoint for the chronic toxicity promulgated methods to detect toxic effects using a two-sample, one-sided hypothesis test (t-test) at two

[1] Tables B-7 through B-18 begin on page B-14.

significance levels, $\alpha = 0.05$ and $\alpha = 0.01$. Data for Method 1009.0 (red macroalga) are not presented, because characterizing method performance using data from only two laboratories and 23 tests is inadvisable.

Table B-14 shows the power and PMSD to be expected for various combinations of (1) number of replicates; (2) k, number of treatments compared with a control; and (3) value of the square root of the error mean square (rEMS) divided by the control mean, when the t-test can be used.

Table B-15 shows the value of PMSD for various combinations of number of replicates, number of treatments compared with a control, and rEMS/(Control Mean). (For definitions and explanations of the terms used here, see Chapters 2 and 3.) This table can be used as a guide to planning the number of replicates needed to achieve a given PMSD. The number of replicates needed can be determined by calculating MSD using the average EMS for a series of tests (at least 20 tests are recommended) and experimenting with various choices of number of replicates (the same number for each concentration and test). This approach is recommended because it uses a sample of test EMSs specific to a particular laboratory. This approach also reveals variation by test, showing how frequently PMSD exceeds the upper bound in Table 3-6 if the number of replicates is increased.

The number of replicates needed to achieve a given value of PMSD will depend on the variability among replicates (rEMS). Table B-16 shows percentiles of the rEMS divided by the control mean, for each promulgated method for chronic toxicity, pooling all tests available in the WET variability data set. The data for Method 1009.0 (red macroalga, *Champia parvula*) are based on only two laboratories and 23 tests and therefore cannot be considered representative.

Table B-15 can be used to infer the number of replicates needed to make the MSD a certain percentage of the control mean (25 percent and 33 percent are used here) for any particular value of rEMS. Table B-17 shows the number of replicates needed to do the same for the 90^{th} and 85^{th} percentiles of rEMS found in Table B-16, in which three or four treatments are compared to a control. These percentiles represent rather extreme examples of imprecision. The precision achieved in most tests and by most laboratories is within the bounds set by these percentiles. The exact number of replicates was not determined beyond “>15” (*Ceriodaphnia* chronic test).

Table B-17 agrees with conclusions drawn from Table 5-1: For most methods, most laboratories can detect a 33 percent effect most of the time, but many laboratories are unable to detect a 25 percent difference between treatment and control in many tests.

B.5 NOEC for Chronic Toxicity Test Methods (Calculated Using the Most Sensitive Endpoint)

NOEC for chronic toxicity methods is calculated using the most sensitive endpoint in each test (meaning the smallest NOEC among those for the two or three endpoints). Table B-18 shows percentiles of within-laboratory CVs in a format like that for Tables B-1 through B-6, and similar calculations were used.

Table B-7a. Percentiles of Control CV for Sublethal Endpoints of Chronic WET Tests, Using Data Pooled Across All Laboratories and Toxicants[a]

	Test Method					
	1000.0 Fathead Minnow	**1002.0 *Cerio-daphnia***	**1003.0 Green Alga**	**1004.0 Sheepshead Minnow**	**1006.0 Inland Silverside**	**1007.0 Mysid (*A. bahia*)**
No. of tests	205	393	85	57	193	130
No. of labs	19	33	9	5	16	10
Endpoint[b]	G	R	G	G	G	G
Percentile	**Control CV**					
5%	0.03	0.08	0.03	0.03	0.03	0.07
10%	0.04	0.09	0.03	0.03	0.04	0.09
15%	0.05	0.10	0.04	0.04	0.05	0.09
20%	0.06	0.11	0.05	0.04	0.06	0.10
25%	0.06	0.12	0.05	0.04	0.06	0.11
50%	0.10	0.20	0.08	0.07	0.10	0.15
75%	0.14	0.33	0.12	0.09	0.14	0.20
80%	0.16	0.36	0.14	0.09	0.14	0.22
85%	0.17	0.39	0.16	0.10	0.16	0.25
90%	0.20	0.42	0.17	0.13	0.18	0.28
95%	0.23	0.52	0.18	0.17	0.23	0.37

[a] Methods in Table B-1 having fewer than three laboratories or fewer than 20 tests are not shown here because so few results may not be representative of method performance.
[b] G = growth, R = reproduction

Table B-7b. Percentiles of Control CV for Endpoints of Chronic WET Tests, Using Data Pooled Across All Laboratories and Toxicants (West Coast Methods)[a]

	Test Method					
	1013.0 Mussel Embryo-Larval Survival & Development	**1014.0 Red Abalone Larval Development**	**1016.0 Sea Urchin Fertilization**	**1017.0 Sand Dollar Fertilization**	**1018.0 Giant Kelp Germination & Germ-Tube Length**	**1018.0 Giant Kelp Germination & Germ-Tube Length**
No. of tests	34	137	159	67	159	159
No. of labs	3	10	11	7	11	11
Endpoint[b]	S	L	F	F	G_e	L
Percentile	**Control CV**					
5%	0.01	0.01	0.01	0.01	0.01	0.02
10%	0.01	0.01	0.01	0.01	0.02	0.03
15%	0.01	0.01	0.01	0.02	0.02	0.03
20%	0.01	0.01	0.02	0.02	0.02	0.04
25%	0.01	0.02	0.02	0.03	0.02	0.05
50%	0.02	0.03	0.04	0.04	0.04	0.07
75%	0.04	0.05	0.07	0.06	0.06	0.09
80%	0.05	0.05	0.08	0.07	0.06	0.11
85%	0.06	0.05	0.10	0.08	0.07	0.11
90%	0.07	0.06	0.12	0.08	0.08	0.12
95%	0.07	0.08	0.18	0.12	0.10	0.14

[a] Methods in Table B-1 having fewer than three laboratories or fewer than 20 tests are not shown here because so few results may not be representative of method performance.

[b] G_e = germination, F = fertilization, L = length, S = survival

Table B-7c. Percentiles of Control CV for Survival Endpoint of Acute WET Tests, Using Data Pooled Across All Laboratories and Toxicants

	Test Method							
	2000.0 Fathead Minnow	**2002.0 *Cerio-daphnia***	**2004.0 Sheepshead Minnow**	**2006.0 Inland Silverside**	**2007.0 Mysid (*A. bahia*)**	**2011.0 Mysid (*H. costata*)**	**2021.0 Daphnia (*D. magna*)**	**2022.0 Daphnia (*D. pulex*)**
No. of tests	217	241	65	48	32	14	48	57
No. of labs	20	23	5	5	3	2	5	6
Percentile	**Control CV**							
5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50%	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00
75%	0.00	0.00	0.00	0.07	0.07	0.07	0.00	0.00
80%	0.00	0.00	0.00	0.07	0.07	0.07	0.00	0.00
85%	0.07	0.00	0.00	0.07	0.07	0.07	0.10	0.07
90%	0.07	0.11	0.00	0.08	0.08	0.07	0.11	0.11
95%	0.11	0.11	0.06	0.10	0.11	0.10	0.12	0.11

Table B-8a. Percentiles of PMSD for Sublethal Endpoints of Chronic WET Tests, Using Data Pooled Across All Laboratories and Toxicants[a,b]

	Test Method					
	1000.0 Fathead Minnow	**1002.0 *Cerio-daphnia***	**1003.0 Green Alga**	**1004.0 Sheepshead Minnow**	**1006.0 Inland Silverside**	**1007.0 Mysid (*A. bahia*)**
No. of tests	205	393	85	57	193	130
No. of labs	19	33	9	5	16	10
Endpoint[c]	G	R	G	G	G	G
Percentile	**PMSD**					
5%	6.8	10	8.2	5.5	10	10
10%	9	11	9.3	6.3	12	12
15%	11	13	10	6.8	12	14
20%	13	15	11	7.9	13	16
25%	14	16	11	8.4	14	16
50%	20	23	14	13	18	20
75%	25	30	19	18	25	25
80%	28	31	20	19	27	26
85%	29	33	21	21	31	28
90%	35	37	23	23	35	32
95%	44	43	27	26	41	34

[a] PMSD = Percent MSD [100×MSD/(Control Mean)]
[b] Methods in Table B-1 having fewer than three laboratories or fewer than 20 tests are not shown here because so few results may not be representative of method performance.
[c] G = growth, R = reproduction

Table B-8b. Percentiles of PMSD for Endpoints of Chronic WET Tests, Using Data Pooled Across All Laboratories and Toxicants (West Coast Methods)[a, b]

	Test Method					
	1013.0 Mussel Embryo-Larval Survival & Development	1014.0 Red Abalone Larval Development	1016.0 Sea Urchin Fertilization	1017.0 Sand Dollar Fertilization	1018.0 Giant Kelp Germination & Germ-Tube Length	1018.0 Giant Kelp Germination & Germ-Tube Length
No. of tests	34	137	159	67	159	159
No. of labs	3	10	11	7	11	11
Endpoint[c]	S	L	F	F	G_e	L
Percentile	**PMSD**					
5%	3.9	3.1	3.7	6.5	5.7	6.6
10%	5.5	3.8	5.1	6.9	6.5	7.9
15%	6.2	4.6	6.5	8.0	7.0	8.8
20%	7.1	5.0	7.3	8.5	7.4	9.2
25%	8.5	5.3	8.1	9.0	8.2	9.6
50%	11	7.9	12	12	10	11
75%	16	12	18	17	14	15
80%	19	13	19	19	15	16
85%	20	15	21	21	17	18
90%	42	16	25	26	18	21
95%	49	20	29	30	20	24

[a] PMSD = Percent MSD [100×MSD/(Control Mean)]
[b] Methods in Table B-1 having fewer than three laboratories or fewer than 20 tests are not shown here because so few results may not be representative of method performance.
[c] G_e = germination, F = fertilization, L = length, S = survival

Table B-8c. Percentiles of PMSD for Survival Endpoint of Acute WET Tests, Using Data Pooled Across All Laboratories and Toxicants[a]

	Test Method							
	2000.0 Fathead Minnow	**2002.0 *Cerio-daphnia***	**2004.0 Sheepshead Minnow**	**2006.0 Inland Silverside**	**2007.0 Mysid (*A. bahia*)**	**2011.0 Mysid (*H. costata*)**	**2021.0 *Daphnia* (*D. magna*)**	**2022.0 *Daphnia* (*D. pulex*)**
No. of tests	217	241	65	48	32	14	48	57
No. of labs	20	23	5	5	3	2	5	6
Percentile	**PMSD**							
5%	0	4.6	0	4.5	3.9	14	4.5	4.3
10%	4.2	5.0	0	7.0	5.1	18	5.3	5.8
15%	5.0	5.6	0	8.9	6.9	21	6.4	6.8
20%	6.6	5.9	0	10	8.4	22	6.9	7.5
25%	7.4	7.1	6.1	12	8.9	23	8.4	8.3
50%	13	11	16	20	15	30	13	14
75%	21	16	32	26	23	38	19	20
80%	23	18	36	29	24	40	20	21
85%	26	19	49	36	24	42	20	22
90%	30	21	55	41	26	47	23	23
95%	51	25	67	46	33	58	27	27

[a] PMSD = Percent MSD [100×MSD/(Control Mean)]

Table B-9. Test Method 1000.0, Fathead Minnow Chronic Toxicity Test, Growth Endpoint: Power and Effect Size Achieved

Lab	No. of Tests	No. of Reps Per Test	Average Control Mean	Average Control Std Dev	Square Root of Variance of Control Mean	Square Root of Average EMS	Average PMSD	Power of Hypothesis Test (2-sample, 1-sided t-test)							
								α = 0.05				α = 0.01			
								N (Reps)	Delta	100×Delta/ Mean	Power	N (Reps)	Delta	100×Delta/ Mean	Power
1	9	4	0.38	0.040	0.081	0.043	19	4	0.09	23	0.85	6	0.12	33	0.48
2	13	4	0.32	0.013	0.028	0.013	6	2	0.03	8	1.00	3	0.04	12	1.00
3	11	3	0.55	0.066	0.117	0.069	25	5	0.17	31	0.62	7	0.26	48	0.13
4	18	4	0.45	0.051	0.107	0.066	21	6	0.13	30	0.67	9	0.19	42	0.25
5	8	4	0.41	0.041	0.115	0.064	26	6	0.13	31	0.63	10	0.18	44	0.21
6	10	3	0.60	0.081	0.189	0.082	28	5	0.20	34	0.54	8	0.31	52	0.10
7	7	4	0.39	0.063	0.064	0.073	31	9	0.15	38	0.47	14	0.21	54	0.12
8	20	4	0.55	0.053	0.109	0.065	17	4	0.13	24	0.82	7	0.19	34	0.43
9	5	4	0.46	0.054	0.217	0.044	17	3	0.09	20	0.93	5	0.13	28	0.68
10	11	3 to 4	0.34	0.047	0.042	0.043	20	5	0.11	32	0.60	7	0.16	49	0.13
11	11	3 to 4	0.54	0.074	0.101	0.084	21	6	0.21	39	0.44	10	0.32	59	0.08
12	11	4	0.59	0.083	0.142	0.076	20	5	0.15	26	0.77	7	0.22	37	0.35
13	10	4	0.42	0.046	0.080	0.044	16	4	0.09	21	0.90	6	0.13	30	0.58
14	11	3 to 4	0.39	0.055	0.063	0.063	26	7	0.16	41	0.40	11	0.24	63	0.07
15	8	3 to 4	0.48	0.048	0.108	0.051	18	4	0.13	27	0.76	6	0.19	41	0.22
16	11	3 to 4	0.35	0.041	0.056	0.052	23	6	0.13	37	0.48	9	0.20	57	0.08
17	6	3	0.40	0.050	0.055	0.098	31	13	0.25	62	0.21	22	0.38	95	0.03
18	20	4	0.40	0.061	0.095	0.064	27	6	0.13	32	0.60	10	0.18	46	0.19
19	6	4	0.54	0.061	0.177	0.060	19	4	0.12	22	0.87	6	0.17	32	0.51

NOTE: Column “N (Reps)” shows the number of replicates needed to detect a 25 percent difference from control with power 0.8, given the observed averages for EMS and control mean. Column “Delta” gives the effect size of the endpoint in milligrams that can be detected with power 0.8, given the observed averages for EMS and control mean. Column “100×Delta/Mean” gives the effect size as a percent of the control mean. Column “Power” gives the power to detect a 25 percent difference from control, given the observed averages for EMS and control mean. PMSD = 100 × MSD / (Control Mean); EMS = error mean square.

Table B-10. Test Method 1002.0, *Ceriodaphnia* Chronic Toxicity Test, Reproduction Endpoint: Power and Effect Size Achieved

								Power of Hypothesis Test (2-sample, 1-sided t-test)							
								α = 0.05				α = 0.01			
Lab	No. of Tests	No. of Reps Per Test	Average Control Mean	Average Control Std Dev	Square Root of Variance of Control Mean	Square Root of Average EMS	Average PMSD	N (Reps)	Delta	100×Delta/ Mean	Power	N (Reps)	Delta	100×Delta/ Mean	Power
1	11	10	34	3.3	2.9	4.6	13	5	5.3	16	0.99	8	7.0	21	0.94
2	9	10	25	7.2	2.6	7.1	29	18	8.2	33	0.59	28	10.8	44	0.28
3	13	10	17	2.6	1.4	3.6	18	10	4.1	24	0.82	16	5.4	32	0.55
4	20	7 to 10	28	8.8	9.5	7.2	25	15	10.2	37	0.51	24	13.6	49	0.20
5	15	10 to 15	19	6.1	4.0	6.6	32	24	7.7	40	0.46	39	10.1	52	0.19
6	20	9 to 10	22	8.5	3.4	7.8	32	26	9.5	44	0.40	42	12.6	58	0.15
7	20	9 to 10	34	11.8	9.7	10.3	31	19	12.7	37	0.50	31	16.8	49	0.21
8	18	10	22	8.6	6.3	7.4	31	23	8.6	39	0.48	37	11.3	51	0.20
9	13	10	25	4.9	3.0	4.8	17	8	5.6	22	0.88	13	7.3	29	0.66
10	12	10	20	2.1	0.8	2.4	12	4	2.8	14	1.00	6	3.6	18	0.98
11	13	10	17	1.5	0.5	3.2	15	8	3.7	21	0.90	13	4.8	28	0.68
12	12	10	31	4.8	2.8	5.0	15	6	5.8	19	0.95	10	7.6	24	0.82
13	8	10	24	5.1	2.5	5.3	22	11	6.2	25	0.79	17	8.1	33	0.51
14	8	10	24	9.2	5.0	6.7	27	17	7.8	33	0.59	28	10.2	43	0.28
15	12	10	18	5.2	2.7	4.8	24	15	5.6	31	0.65	24	7.4	40	0.34
16	20	10	21	5.4	4.6	4.9	22	12	5.7	27	0.74	19	7.5	36	0.44
17	10	9 to 10	24	6.1	4.5	6.9	29	18	8.5	35	0.54	29	11.2	47	0.23
18	10	10	20	5.8	3.7	5.5	24	15	6.4	31	0.64	25	8.4	41	0.32
19	6	9 to 10	23	10.9	3.9	8.4	36	28	10.3	45	0.38	45	13.6	60	0.13
20	12	10	23	3.3	4.7	4.9	21	10	5.7	24	0.81	16	7.5	32	0.54
21	9	10	28	5.3	3.0	6.0	20	11	6.9	25	0.79	17	9.1	33	0.51
22	10	10	17	4.5	2.2	4.9	26	17	5.7	33	0.59	28	7.6	43	0.28
23	9	9 to 10	27	6.9	3.6	7.4	27	16	9.1	33	0.58	25	12.0	44	0.27
24	10	10	18	4.4	1.4	4.5	23	13	5.3	29	0.70	21	6.9	38	0.39
25	12	10	20	6.4	3.6	6.0	30	19	7.0	35	0.55	30	9.2	46	0.25
26	12	10	27	4.4	3.2	4.2	14	6	4.9	18	0.96	10	6.5	24	0.84
27	10	10	21	6.0	4.0	6.1	27	19	7.0	34	0.56	30	9.3	45	0.26
28	6	10	20	6.1	5.2	4.7	23	12	5.5	27	0.74	20	7.3	36	0.43
29	14	10	31	5.6	3.0	5.9	19	9	6.8	22	0.87	14	9.0	29	0.64
30	5	10	16	4.7	0.3	4.9	28	20	5.7	36	0.53	32	7.4	47	0.24
31	12	10	24	5.4	5.9	6.1	25	14	7.1	30	0.67	23	9.3	39	0.35
32	4	10	32	5.9	6.3	5.6	17	8	6.5	21	0.91	12	8.6	27	0.72
33	18	10	24	6.9	5.6	6.8	28	17	7.9	32	0.61	27	10.3	42	0.30

NOTE: See note at bottom of Table B-9.

Table B-11. Test Method 1004.0, Sheepshead Minnow Chronic Toxicity Test, Growth Endpoint: Power and Effect Size Achieved

Lab	No. of Tests	No. of Reps Per Test	Average Control Mean	Average Control Std Dev	Square Root of Variance of Control Mean	Square Root of Average EMS	Average PMSD	Power of Hypothesis Test (2-sample, 1-sided t-test)							
								α = 0.05				α = 0.01			
								N (Reps)	Delta	100×Delta/ Mean	Power	N (Reps)	Delta	100×Delta/ Mean	Power
1	12	4	0.88	0.040	0.11	0.037	6.6	2	0.08	8.6	1.00	3	0.11	12	1.00
2	11	4	0.68	0.051	0.11	0.071	16	4	0.14	21	0.90	6	0.20	30	0.59
3	16	4	0.65	0.088	0.091	0.084	20	5	0.17	26	0.77	7	0.24	37	0.34
4	14	4	1.00	0.074	0.13	0.076	12	3	0.15	15	0.98	4	0.22	22	0.91
5	4	4	0.86	0.048	0.12	0.066	11	3	0.13	16	0.98	4	0.19	22	0.90

NOTE: See note at bottom of Table B-9.

Table B-12. Test Method 1006.0, Inland Silverside Chronic Toxicity Test: Power and Effect Size Achieved

Lab	No. of Tests	No. of Reps Per Test	Average Control Mean	Average Control Std Dev	Square Root of Variance of Control Mean	Square Root of Average EMS	Average PMSD	Power of Hypothesis Test (2-sample, 1-sided t-test)							
								α = 0.05				α = 0.01			
								N (Reps)	Delta	100×Delta/ Mean	Power	N (Reps)	Delta	100×Delta/ Mean	Power
1	10	4	2.3	0.18	0.58	0.26	18	4	0.53	23	0.86	6	0.75	32	0.50
2	15	4	0.94	0.10	0.24	0.17	20	8	0.34	36	0.52	12	0.48	51	0.15
3	19	4	2.1	0.24	0.86	0.27	19	5	0.54	25	0.79	7	0.76	36	0.38
4	12	3	1.4	0.20	0.56	0.22	32	7	0.56	42	0.40	11	0.86	63	0.07
5	6	3 to 4	1.8	0.25	0.57	0.43	31	12	1.07	59	0.23	20	1.6	90	0.04
6	19	4	0.85	0.11	0.23	0.10	20	4	0.20	24	0.83	7	0.29	34	0.43
7	20	3 to 4	1.4	0.15	0.53	0.31	31	11	0.79	56	0.24	18	1.2	86	0.04
8	4	4 to 5	1.1	0.10	0.20	0.11	15	4	0.23	21	0.91	5	0.33	29	0.62
9	20	4	2.4	0.23	0.47	0.25	17	4	0.51	22	0.89	6	0.73	31	0.56
10	20	3 to 4	0.91	0.088	0.35	0.11	22	4	0.27	30	0.65	7	0.42	46	0.15
11	9	4	1.2	0.13	0.19	0.11	14	3	0.22	18	0.96	5	0.31	25	0.79
12	7	4	2.1	0.22	0.38	0.25	17	4	0.50	24	0.84	6	0.72	34	0.45
13	14	4	0.76	0.095	0.12	0.11	22	5	0.22	28	0.70	8	0.31	40	0.27
14	5	4	1.5	0.12	0.33	0.12	13	3	0.25	17	0.97	4	0.35	24	0.84
15	8	4	0.77	0.10	0.22	0.12	25	6	0.24	31	0.64	9	0.34	44	0.22
16	5	3	1.2	0.11	0.20	0.14	20	4	0.35	30	0.67	6	0.53	45	0.16

NOTE: See note at bottom of Table B-9.

Table B-13. Test Method 1007.0, Mysid Chronic Toxicity Test, Growth Endpoint: Power and Effect Size Achieved

Lab	No. of Tests	No. of Reps Per Test	Average Control Mean	Average Control Std Dev	Square Root of Variance of Control Mean	Square Root of Average EMS	Aver-age PMSD	Power of Hypothesis Test (2-sample, 1-sided t-test) α = 0.05 N (Reps)	Delta	100×Delta/ Mean	Power	α = 0.01 N (Reps)	Delta	100×Delta/ Mean	Power
1	18	8	0.25	0.040	0.042	0.041	17	7	0.054	22	0.89	11	0.072	29	0.66
2	19	8	0.37	0.15	0.13	0.11	25	20	0.15	41	0.44	33	0.20	54	0.16
3	7	4	0.36	0.042	0.065	0.047	21	5	0.094	26	0.77	7	0.13	37	0.35
4	12	8	0.25	0.044	0.035	0.13	37	58	0.18	70	0.21	94	0.23	94	0.06
5	10	8	0.37	0.073	0.049	0.075	22	9	0.098	26	0.76	15	0.13	35	0.45
6	14	8	0.23	0.034	0.059	0.040	20	7	0.053	22	0.87	11	0.070	30	0.62
7	18	8	0.28	0.075	0.056	0.067	26	13	0.089	32	0.62	20	0.12	42	0.30
8	12	8	0.30	0.048	0.070	0.053	19	8	0.070	23	0.85	12	0.093	31	0.58
9	16	8	0.38	0.041	0.048	0.060	16	7	0.079	21	0.90	10	0.11	28	0.68
10	4	8	0.30	0.041	0.018	0.047	14	6	0.061	21	0.91	10	0.081	27	0.71

NOTE: See note at bottom of Table B-9.

Table B-14. Power to Detect a 25% Difference Between Two Means in a Two-sample, One-sided Test (continued)

N (Reps)	k	df	rEMS / Control Mean = 0.10 PMSD	Power With α= 0.05	α= 0.05/k	rEMS / Control Mean = 0.20 PMSD	Power With α= 0.05	α= 0.05/k	rEMS / Control Mean =0.30 PMSD	Power With α= 0.05	α= 0.05/k	rEMS / Control Mean = 0.40 PMSD	Power With α= 0.05	α= 0.05/k
3	2	4	21	0.80	0.66	43	0.29	0.17	64	0.16	0.09	85	0.12	0.07
3	3	6	21	0.80	0.68	42	0.29	0.18	63	0.16	0.10	84	0.12	0.07
3	4	8	21	0.80	0.68	42	0.29	0.18	63	0.16	0.10	83	0.12	0.07
3	5	10	21	0.80	0.68	42	0.29	0.18	63	0.16	0.10	84	0.12	0.07
4	2	6	17	0.92	0.86	33	0.43	0.29	50	0.24	0.15	66	0.17	0.10
4	3	9	17	0.92	0.86	34	0.43	0.28	50	0.24	0.14	67	0.17	0.09
4	4	12	17	0.92	0.85	34	0.43	0.27	51	0.24	0.13	68	0.17	0.09
4	5	15	17	0.92	0.84	35	0.43	0.26	52	0.24	0.13	69	0.17	0.08
5	2	8	14	0.97	0.94	28	0.55	0.41	42	0.30	0.20	56	0.20	0.13
5	3	12	14	0.97	0.93	29	0.55	0.38	43	0.30	0.18	58	0.20	0.12
5	4	16	15	0.97	0.93	30	0.55	0.36	44	0.30	0.17	59	0.20	0.11
5	5	20	15	0.97	0.92	30	0.55	0.35	45	0.30	0.16	60	0.20	0.10
6	2	10	12	0.98	0.97	25	0.63	0.51	37	0.36	0.25	50	0.24	0.16
6	3	15	13	0.98	0.97	26	0.63	0.47	39	0.36	0.22	52	0.24	0.14
6	4	20	13	0.98	0.96	27	0.63	0.45	40	0.36	0.20	53	0.24	0.12
6	5	25	14	0.98	0.96	27	0.63	0.43	41	0.36	0.19	54	0.24	0.12

Table B-14. Power to Detect a 25% Difference Between Two Means in a Two-sample, One-sided Test

N (Reps)	k	df	rEMS / Control Mean = 0.10			rEMS / Control Mean = 0.20			rEMS / Control Mean =0.30			rEMS / Control Mean = 0.40		
			PMSD	Power With α= 0.05	Power With α= 0.05/k	PMSD	Power With α= 0.05	Power With α= 0.05/k	PMSD	Power With α= 0.05	Power With α= 0.05/k	PMSD	Power With α= 0.05	Power With α= 0.05/k
7	5	30	12	0.99	0.98	25	0.71	0.50	37	0.41	0.23	50	0.28	0.13
8	2	14	10	1.00	0.99	21	0.76	0.66	31	0.46	0.34	42	0.31	0.21
8	3	21	11	1.00	0.99	22	0.76	0.62	33	0.46	0.31	44	0.31	0.18
8	4	28	11	1.00	0.99	23	0.76	0.59	34	0.46	0.28	45	0.31	0.16
8	5	35	12	1.00	0.99	23	0.76	0.57	35	0.46	0.26	46	0.31	0.15
9	2	16	10	1.00	1.00	19	0.81	0.72	29	0.51	0.39	39	0.34	0.24
9	3	24	10	1.00	1.00	20	0.81	0.68	31	0.51	0.35	41	0.34	0.21
9	4	32	11	1.00	1.00	21	0.81	0.65	32	0.51	0.32	42	0.34	0.18
9	5	40	11	1.00	1.00	22	0.81	0.63	33	0.51	0.30	44	0.34	0.17
10	2	18	9	1.00	1.00	18	0.85	0.77	27	0.55	0.43	36	0.37	0.26
10	3	27	10	1.00	1.00	19	0.85	0.73	29	0.55	0.39	39	0.37	0.23
10	4	36	10	1.00	1.00	20	0.85	0.71	30	0.55	0.36	40	0.37	0.21
10	5	45	10	1.00	1.00	21	0.85	0.69	31	0.55	0.33	41	0.37	0.19
11	2	20	9	1.00	1.00	17	0.88	0.81	26	0.59	0.47	35	0.40	0.29
11	3	30	9	1.00	1.00	18	0.88	0.78	27	0.59	0.42	37	0.40	0.25
11	4	40	10	1.00	1.00	19	0.88	0.75	29	0.59	0.39	38	0.40	0.23
11	5	50	10	1.00	1.00	20	0.88	0.73	29	0.59	0.37	39	0.40	0.21
12	2	22	8	1.00	1.00	16	0.90	0.85	25	0.63	0.51	33	0.43	0.32
12	3	33	9	1.00	1.00	17	0.90	0.82	26	0.63	0.46	35	0.43	0.27
12	4	44	9	1.00	1.00	18	0.90	0.79	27	0.63	0.43	36	0.43	0.25
12	5	55	9	1.00	1.00	19	0.90	0.78	28	0.63	0.40	37	0.43	0.23
13	2	24	8	1.00	1.00	16	0.92	0.87	24	0.66	0.55	32	0.45	0.34
13	3	36	8	1.00	1.00	17	0.92	0.85	25	0.66	0.50	33	0.45	0.30
13	4	48	9	1.00	1.00	17	0.92	0.83	26	0.66	0.46	35	0.45	0.27
13	5	60	9	1.00	1.00	18	0.92	0.81	27	0.66	0.44	36	0.45	0.25
14	2	26	8	1.00	1.00	15	0.94	0.90	23	0.69	0.58	30	0.48	0.37
14	3	39	8	1.00	1.00	16	0.94	0.88	24	0.69	0.53	32	0.48	0.32
14	4	52	8	1.00	1.00	17	0.94	0.86	25	0.69	0.50	33	0.48	0.29
14	5	65	9	1.00	1.00	17	0.94	0.84	26	0.69	0.47	34	0.48	0.27
15	2	28	7	1.00	1.00	15	0.95	0.92	22	0.72	0.61	29	0.50	0.39
15	3	42	8	1.00	1.00	15	0.95	0.90	23	0.72	0.56	31	0.50	0.34
15	4	56	8	1.00	1.00	16	0.95	0.88	24	0.72	0.53	32	0.50	0.31
15	5	70	8	1.00	1.00	17	0.95	0.87	25	0.72	0.50	33	0.50	0.29

NOTE: Power is reported for tests with two values of α, 0.05 and 0.05/k. Power for Dunnett's multiple comparison test will fall between these two values. All numbers have been rounded to two significant figures. The number of treatments tested (k) and used to calculate EMS and MSD for a sublethal endpoint will vary depending on the NOEC for survival. k = number of treatments in Dunnett's test; df = degrees of freedom; PMSD = 100 × MSD / (Control Mean); EMS = error mean square; rEMS = square root of the error mean square.

Table B-15. Values of PMSD in Dunnett's Test in Relation to the Square Root of the Error Mean Square (rEMS) for the Test

Reps	k	df	d	Value of PMSD When rEMS / (Control Mean) Equals These Values			
				0.1	0.2	0.3	0.4
3	2	4	2.61	21	43	64	85
4	2	6	2.34	17	33	50	66
5	2	8	2.22	14	28	42	56
6	2	10	2.15	12	25	37	50
7	2	12	2.11	11	23	34	45
8	2	14	2.08	10	21	31	42
9	2	16	2.06	10	19	29	39
10	2	18	2.04	9	18	27	37
11	2	20	2.03	9	17	26	35
12	2	22	2.02	8	16	25	33
13	2	24	2.01	8	16	24	32
14	2	26	2.00	8	15	23	30
15	2	28	1.99	7	15	22	29
3	3	6	2.56	21	42	63	84
4	3	9	2.37	17	34	50	67
5	3	12	2.29	14	29	43	58
6	3	15	2.24	13	26	39	52
7	3	18	2.21	12	24	35	47
8	3	21	2.19	11	22	33	44
9	3	24	2.17	10	20	31	41
10	3	27	2.16	10	19	29	39
11	3	30	2.15	9	18	27	37
12	3	33	2.14	9	17	26	35
13	3	36	2.13	8	17	25	33
14	3	39	2.13	8	16	24	32
15	3	42	2.12	8	15	23	31
3	4	8	2.55	21	42	63	83
4	4	12	2.41	17	34	51	68
5	4	16	2.34	15	30	44	59
6	4	20	2.30	13	27	40	53
7	4	24	2.28	12	24	37	49
8	4	28	2.26	11	23	34	45
9	4	32	2.25	11	21	32	42
10	4	36	2.24	10	20	30	40
11	4	40	2.23	10	19	29	38
12	4	44	2.22	9	18	27	36

Table B-15. Values of PMSD in Dunnett's Test in Relation to the Square Root of the Error Mean Square (rEMS) for the Test

Reps	k	df	d	Value of PMSD When rEMS / (Control Mean) Equals These Values			
				0.1	0.2	0.3	0.4
13	4	48	2.22	9	17	26	35
14	4	52	2.21	8	17	25	33
15	4	56	2.21	8	16	24	32
3	5	10	2.56	21	42	63	84
4	5	15	2.44	17	35	52	69
5	5	20	2.39	15	30	45	60
6	5	25	2.36	14	27	41	54
7	5	30	2.34	12	25	37	50
8	5	35	2.32	12	23	35	46
9	5	40	2.31	11	22	33	44
10	5	45	2.30	10	21	31	41
11	5	50	2.29	10	20	29	39
12	5	55	2.29	9	19	28	37
13	5	60	2.28	9	18	27	36
14	5	65	2.28	9	17	26	34
15	5	70	2.28	8	17	25	33

NOTE: The number of treatments tested (k) and used to calculate EMS and MSD for a sublethal endpoint will vary depending on the NOEC for survival. k = number of treatments in Dunnett's test; df = degrees of freedom; d = Dunnett's statistic ($\alpha = 0.05$); PMSD = 100 × MSD / (Control Mean); EMS = error mean square; rEMS = square root of the error mean square.

Table B-16. Percentiles of the rEMS/Control Mean, for the Growth or Reproduction Endpoint of Chronic WET Tests, Using Data Pooled Across All Laboratories and Toxicants[a]

	Test Method						
	1000.0 Fathead Minnow	**1002.0 *Cerio-daphnia***	**1003.0 Green Alga**	**1004.0 Sheepshead Minnow**	**1006.0 Inland Silverside**	**1007.0 Mysid (*A. bahia*)**	**1009.0 Red Macroalga**
No. of tests	206	393	85	57	193	130	23
No. of labs	19	33	9	5	16	10	2
Endpoint	G	R	G	G	G	G	R
Percentile	**rEMS/Control Mean**						
25%	0.09	0.17	0.06	0.05	0.09	0.15	0.11
50%	0.12	0.24	0.08	0.08	0.11	0.18	0.18
75%	0.16	0.31	0.10	0.11	0.15	0.23	0.25
80%	0.17	0.32	0.11	0.12	0.16	0.24	0.26
85%	0.18	0.34	0.12	0.13	0.18	0.27	0.27
90%	0.21	0.39	0.13	0.14	0.21	0.29	0.27
95%	0.26	0.44	0.16	0.15	0.26	0.33	0.34

[a] rEMS = square root of the error mean square
[b] G = growth, R = reproduction

Table B-17. Number of Replicates Needed to Provide PMSD of 25% and 33% for Some Less Precise Tests in Each Chronic Test Method (that is, for 85th and 90th Percentiles from Table B-17) for the Sublethal Endpoints in Table B-16

		rEMS / Control Mean		Number of Replicates to Make PMSD = 25		Number of Replicates to Make PMSD = 33	
Test Method	**Required No. of Replicates**	**85th Percentile**	**90th Percentile**	**For 85th Percentile**	**For 90th Percentile**	**For 85th Percentile**	**For 90th Percentile**
1000.0 Fathead Minnow	4 (3)	0.18	0.21	6	8 (7)	4	5
1002.0 *Ceriodaphnia*	10	0.34	0.39	19 (17)	24 (22)	11	14 (13)
1003.0 Green Alga	4 (3)	0.12	0.13	4	4	3	3
1004.0 Sheepshead Minnow	4 (3)	0.13	0.14	4	4	3	3
1006.0 Inland Silverside	4 (3)	0.18	0.21	6	8 (7)	4	5
1007.0 Mysid	8	0.27	0.29	12 (11)	14 (13)	7	9 (8)
1009.0 Red Macroalga	4 (3)	0.27	0.27	12 (11)	12 (11)	7	7

NOTE: The number for k = 3 treatments appears in parentheses if it differs from the number needed when four treatments are compared with the control; rEMS = square root of the error mean square; PMSD = percent minimum significant difference.

Table B-18. Percentiles of the Within-Laboratory Values of CV for NOEC (using NOEC for the Most Sensitive Endpoint in Each Test)

Method No.	Method	No. Labs	P10	P25	P50	P75	P90
1000.0	Fathead Minnow Larval Survival & Growth	19	0	0.22	0.31	0.52	0.65
1002.0	*Ceriodaphnia* Survival & Reproduction	33	0.20	0.25	0.35	0.49	0.60
1003.0	Green Alga Growth	9	0.30	0.40	0.46	0.56	0.82
1004.0	Sheepshead Minnow Larval Survival & Growth	5	0.20	0.36	0.38	0.44	0.52
1006.0	Inland Silverside Larval Survival & Growth	16	0.19	0.35	0.46	0.59	0.66
1007.0	Mysid Survival, Growth, & Fecundity	10	0.28	0.32	0.40	0.50	0.60
1009.0	Red Macroalga Reprod	2	0.85	0.85	1.00	1.16	1.16
1010.0	Topsmelt Larval Survival & Growth	1	0.22	0.22	0.22	0.22	0.22
1012.0	Pacific Oyster Embryo-Larval Survival & Dev.	1	0.45	0.45	0.45	0.45	0.45
1013.0	Mussel Embryo-Larval Survival & Dev.	3	0	0	0.39	0.43	0.43
1014.0	Red Abalone Larval Development	10	0.24	0.25	0.29	0.31	0.38
1016.0	Sea Urchin Fertilization[a]	12	0.31	0.40	0.50	0.69	0.76
1017.0	Sand Dollar Fertilization[a]	7	0.40	0.41	0.53	0.75	0.81
1018.0	Giant Kelp Germination & Germ-Tube Length	11	0.33	0.36	0.59	0.68	0.72

[a] These two test species include previous test method procedures (Dinnel 1987, Chapman 1992). However, EPA (USEPA 1995) has standardized these two methods to provide further guidance and therefore minimize within-test variability.